Another nucleic acid amplification technique is nucleic acid sequence-based amplification (NASBA™) which uses reverse transcription and T7 RNA polymerase and incorporates two primers to target its cycling scheme. NASBA™ amplification can begin with either DNA or RNA and finish with either, and amplifies to about 10^8 copies within 60 to 90 minutes.

Alternatively, nucleic acid can be amplified by ligation activated transcription (LAT). LAT works from a single-stranded template with a single primer that is partially single-stranded and partially double-stranded. Amplification is initiated by ligating a cDNA to the promoter oligonucleotide and within a few hours, amplification is about 10^8 to about 10^9 fold. The QB replicase system can be utilized by attaching an RNA sequence called MDV-1 to RNA complementary to a DNA sequence of interest. Upon mixing with a sample, the hybrid RNA finds its complement among the specimen's mRNAs and binds, activating the replicase to copy the tag-along sequence of interest.

Another nucleic acid amplification technique, ligase chain reaction (LCR), works by using two differently labeled halves of a sequence of interest which are covalently bonded by ligase in the presence of the contiguous sequence in a sample, forming a new target. The repair chain reaction (RCR) nucleic acid amplification technique uses two complementary and target-specific oligonucleotide probe pairs, thermostable polymerase and ligase, and DNA nucleotides to geometrically amplify targeted sequences. A 2-base gap separates the oligo probe pairs, and the RCR fills and joins the gap, mimicking normal DNA repair.

Nucleic acid amplification by strand displacement activation (SDA) utilizes a short primer containing a recognition site for *HincII* with short overhang on the 5' end which binds to target DNA. A DNA polymerase fills in the part of the primer opposite the overhang with sulfur-containing adenine analogs. *HincII* is added but only cuts the unmodified DNA strand. A DNA polymerase that lacks 5' exonuclease activity enters at the cite of the nick and begins to polymerize, displacing the initial primer strand downstream and building a new one which serves as more primer.

Mean Arterial Blood Pressures (mmHg) Following IV Citrulline

Treatment Group (n=4)	Pre-dose	1 hour post	2 hours post	3 hours post
Citrulline (600mg/kg)	67.0	67.4	64.8	62.2
Control (saline)	53.2	58.7	55.7	54.7

$p>.05$ at all time points

Pharmacokinetics: Based on the above data, the pharmacokinetics were calculated for both plasma citrulline and arginie levels after the single dose of IV citrulline. Pharmacokinetic data included plasma half-life (t ½), elimination constant (Kel), volume of distribution (Vd), and plasma clearance (CLp).

Plasma citrulline levels rapidly increased and demonstrated a t ½ =1.5 hrs, Kel =.462 hr^{-1}, Vd = 2.25 L, and CLp = 1.05 L/hr. However, the effect of citrulline on plasma arginine was of interest because it is the substrate for NO synthase. The concentration curve of plasma arginine levels is represented in Figure 13. Based on this curve, the pharmacokinetics of plasma arginine are as follows: t ½= 18 hrs; Kel= .039 hr^{-1}; Vd= 2.85 L; CLp= 0.11 L/hr. The long half-life and slow clearance indicates that a single dose of IV citrulline is effective at maintaining increased plasma arginine levels over a fairly long interval without detrimental effects on hemodynamics.

REFERENCES

The references listed below as well as all references cited in the specification are incorporated herein by reference to the extent that they supplement, explain, provide a background for or teach methodology, techniques and/or compositions employed herein.

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